

Comparative Stimulus Properties of Two Fractions of the Coca Leaf (*E. coca*)¹

JOHN A. BEDFORD,² GREGORY L. NAIL, HALA N. ELSOHLY,
MARVIN C. WILSON AND CARLTON E. TURNER

Research Institute of Pharmaceutical Sciences and Department of Pharmacology
School of Pharmacy, The University of Mississippi, University, MS 38677

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BEDFORD, J. A., G. L. NAIL, H. N. ELSOHLY, M. C. WILSON AND C. E. TURNER. *Comparative stimulus properties of two fractions of the coca leaf (E. coca)*. PHARMAC. BIOCHEM. BEHAV. 15(6) 907-909, 1981.—Male and female Wistar rats were trained to discriminate 5.0 mg/kg cocaine from 2.0 ml/kg saline using a two-bar food reinforcement (FR 30) drug discrimination paradigm. Once discrimination behavior had stabilized the subjects were tested (in extinction) with several doses of two different fractions of the coca leaf and four doses of cocaine HCl (1.0, 2.5, 7.5, 10 mg/kg). The fractions were prepared by extracting powdered coca leaves with 95% ethanol and then partitioning the residue between chloroform and water. Two doses of the water fractions (480, 960 mg/kg) and five doses of the chloroform fraction (7.5, 15, 30, 60, 120 mg/kg) were tested. The water fraction was devoid of cocaine while the five doses of the chloroform fraction contained cocaine equivalent to 0.4, 0.83, 1.65, 3.3 and 6.6 mg/kg, respectively, as determined by gas chromatographic analysis. The 2.5, 7.5, and 10.0 mg/kg cocaine doses generalized to cocaine. The 1.0 mg/kg dose of cocaine generalized to saline. The water fraction at 480 mg/kg generalized to saline; however following pretreatment with the 960 mg/kg dose of this fraction, the animals failed to respond. The two largest doses of the chloroform fraction (60 and 120 mg/kg) generalized to cocaine while the other three doses did not. The 7.5 mg/kg dose generalized to saline; the 15 and 30 mg/kg doses engendered an intermediate level of responding on both the cocaine and saline lever.

Coca Cocaine Stimulus properties Rat

A RECENT paper from this laboratory [2] reported the effects of cocaine and two fractions of the coca leaf (*E. coca*) on food intake and locomotor activity. We concluded that the activity of the chloroform fraction might be solely attributable to its cocaine content. In addition, we reported that the water fraction (containing only trace amounts of cocaine) produced a significant reduction in food consumption by rats at the highest dose tested. We further reported that this activity could not be attributed to the small amount of cocaine present in this fraction. In an attempt to determine whether the coca leaf might contain water soluble constituents capable of eliciting cocaine-like effects in experimental animals, these same two fractions were tested in a drug discrimination paradigm. Such a procedure would ascertain whether rats trained to discriminate cocaine from saline would find these fractions to possess stimulus properties that are similar to those of cocaine.

METHOD

Subjects

The subjects were three male and five female Wistar rats (Harlan Industries, Cumberland, IN) weighing between

200-250 g at the start of the experiment. Subject weights were reduced to and maintained at 85% of free-feeding weights for the duration of the experiment. Water was freely available except during experimental sessions. When not in the experimental chambers, subjects were maintained in individual galvanized steel cages. Ambient temperature was maintained at $21 \pm 1^\circ\text{C}$ and the light/dark cycle was 12 hr on, 12 hr off.

Apparatus

Standard operant conditioning chambers (BRS-LVE, Beltsville, MD) measuring 30 cm long and 39 cm high by 39 cm wide were enclosed in sound attenuating enclosures (BRS-LVE). Two rodent response levers (BRS-LVE) were located on one wall 10 cm up from the wire mesh floor and 7.0 cm from the front and rear walls respectively. A food cup was located equidistant between the two levers and 2 cm from the floor. Three jeweled panel lights were located 5 cm above each lever. Ambient illumination was provided by the 28 VDC panel lights located behind a transparent panel near the top of the chamber. Forced air ventilation provided a continuous air exchange and also served as a source of masking noise.

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²Reprint requests should be sent to Dr. John A. Bedford, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.

Drugs and Solutions

Drug solutions were prepared on the morning of use. Cocaine dosages were calculated on the basis of the hydrochloride salt and were prepared using sterile normal saline as the diluent. Cocaine hydrochloride flakes U.S.P. were obtained from Mallinkrodt Chemical Corp. (St. Louis, MO). Coca leaves (*Erythroxylon coca*) obtained from the Tingo Maria area of Peru were powdered using a Wilay mill and extracted by percolation with 95% ethanol. Evaporation of the solvent resulted in a crude ethanol extract. The crude ethanol extract was then partitioned between water and chloroform resulting in two fractions. All water soluble compounds (e.g., water soluble alkaloids, quaternary ammonium compounds, sugars, cyclitols, glycosides, etc.) were contained in the water fraction while the chloroform fraction contained the water insoluble constituents (e.g., alkaloids, terpenes, sterols, fatty acids, etc.). Injections of dosages of the water fraction were prepared using sterile water, while the chloroform fraction was suspended in sterile water using two drops each of Tween 60 and Arlacel per ml of water. Five dosages (7.5, 15, 30, 60 and 120 mg/kg) of the chloroform fraction were tested. The dosages of cocaine HCl tested were 1.0, 2.5, 7.5 and 10 mg/kg. Cocaine content of the two extracts was determined by gas chromatography using a previously reported method [4]. Briefly the conditions were as follows:

Instrument: Dual column Beckman GC equipped with dual ionization detectors.

Column: 8 ft column \times $\frac{1}{8}$ in diameter, packed with 6% OV-1 on chromosorb W100-200 mesh.

Column temperature: Isothermal at 220°C.

Carrier gas: Nitrogen at a flow rate of 25 ml/min.

Internal standard: Androst-4-ene-3,17 dione.

Procedure

Experimental sessions were conducted daily, 5 days per week and were 8 min long. Following initial bar press training, 5 mg/kg cocaine HCl in sterile 0.9% saline or 2 ml/kg saline were administered IP 10 min prior to each session. Reinforcement (45 mg pellets, P.J. Noyes, Lancaster, NH) was contingent upon pressing the lever, appropriate to the type of pretreatment, on any given day. The lever designated the cocaine lever and the lever designated the saline lever were determined randomly for each subject. The pretreatment sequence followed throughout the study, except on probe (extinction) sessions, was as follows: Cocaine-saline-saline-cocaine, saline-cocaine-cocaine-saline, etc. During the initial discrimination training sessions the reinforcement schedule in effect was FR1 (i.e., each bar press on the appropriate lever was reinforced), however the schedule requirement was gradually increased to a final value of FR 30 (i.e., every 30th response on the appropriate lever was reinforced) and maintained at this value throughout the study. Once discrimination behavior had stabilized (stability criterion=80% responding on correct lever) probe tests were begun. Probe test sessions were conducted on Friday of each week. A probe session consisted of pretreating each subject IP (10 min prior to being placed in the chamber) with one of the doses of either cocaine, the chloroform fraction, water fraction or vehicle. Probe doses of each treatment tested were as follows: Cocaine, 1.0, 2.5, 7.5 and 10 mg/kg; chloroform fraction, 7.5, 15, 30, 60 and 120 mg/kg (which contained 0.4, 0.83, 1.65, 3.3 and 6.6 mg/kg of cocaine, respectively); water fraction, 480 and 960 mg/kg. Each dose of

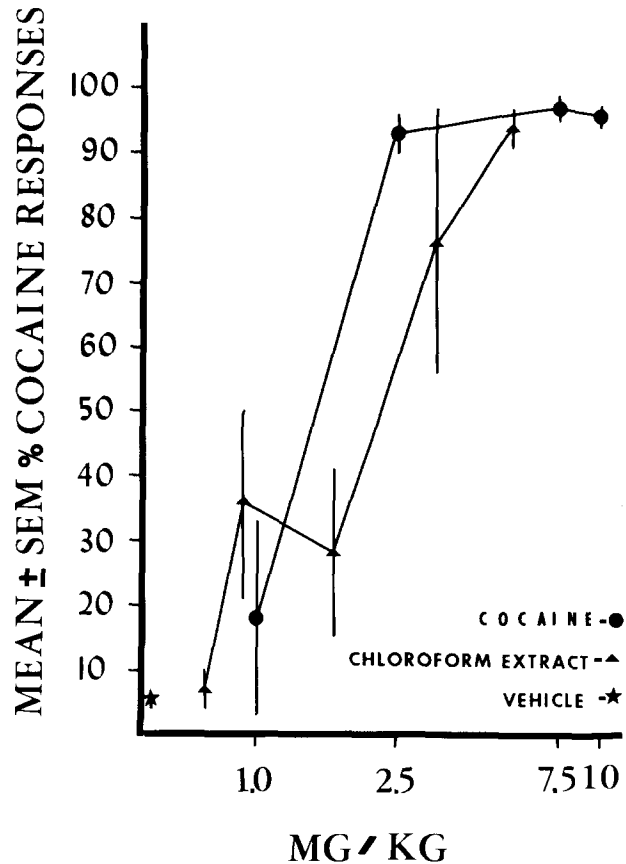


FIG. 1. Results of generalization tests conducted with the chloroform fraction of the coca extract and cocaine. Data point represent the mean percent response on the cocaine lever of eight animals during probe tests. The vertical lines represent the standard error of measurement. The chloroform extract is plotted on the basis of its cocaine content.

each treatment was tested once in separately randomized sequences. During probe sessions the subjects were allowed to respond until 90 responses had occurred on one bar, after which the subject was immediately removed from the chamber. No reinforcement occurred during the probe sessions.

RESULTS

Figure 1 presents the results of the probe tests conducted with cocaine and the chloroform fraction. The 2.5, 7.5 and 10 mg/kg doses of cocaine generalized to cocaine with greater than 90% of the responses following pretreatment with these doses occurring on the cocaine lever. The 1.0 mg/kg dose of cocaine generalized to saline with approximately 94% of the responses occurring on the saline lever. The 480 mg/kg dose of the water fraction generalized to saline; however, following pretreatment with the 960 mg/kg dose, the subjects failed to respond. The highest doses (60 and 120 mg/kg) of the chloroform fraction generalized to cocaine with 75 and 94% of the responses occurring on the cocaine lever. The 7.5 mg/kg dose of this fraction generalized to saline with greater

than 90% of the responses occurring on the saline lever. The 15 and 30 mg/kg doses of the chloroform fraction engendered 37% and 28% responding, respectively, on the cocaine lever.

DISCUSSION

The results presented in the present study support and extend previously published reports on the behavioral effects of fractions of the coca leaf. In a recent report [5] the chloroform fraction was shown to produce typical cocaine-like effects on both fixed-interval and fixed-ratio food reinforced behavior in rats. In the current report, the stimulus properties of the chloroform extract appeared to be entirely a reflection of the cocaine content. This conclusion is supported by the finding that very similar generalization gradients existed between cocaine, and dosages of the chloroform fraction containing equal amounts of cocaine.

In another report [3] large dosages, of the water fraction (480-960 mg/kg) were shown to produce cocaine-like effects, but only on fixed-ratio behavior (i.e., decrease in response rate). In subjects maintained on a fixed-interval schedule similar dosages of this fraction produced a decrease in response rate. Unlike cocaine, no increase in response rates occurred with any dosage. The results obtained with the water fraction suggest the presence of other behaviorally active constituents whose stimulus properties are somewhat different than those of cocaine. However, further testing of doses 480 and 960 mg/kg are required before a definitive

statement concerning the stimulus properties of this fraction can be made. In a recent report [1], the water fraction was further partitioned between butanol and water and these resultant two fractions were tested for their effects on food consumption and locomotor activity. Both of these fractions produced a decrease in food consumption however, without altering locomotor activity. The data from the present study coupled with the recent reports discussed above clearly demonstrate that the activity observed with the coca leaf cannot be wholly attributed to the cocaine content of the leaf.

In summary, the suppression of any responding by the highest dose of the water fraction and the lack of generalization at the lower dose strongly suggests that the coca plant contains constituents in addition to cocaine that are biologically and/or behaviorally active. However, this activity is definitely qualitatively different from cocaine. These various coca fractions are currently being subjected to column chromatography, which will allow us to further isolate the constituents in the plant that are responsible for the observed effects.

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